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Artocarpus lakoocha roxb.: An untapped bioresource of resveratrol from North East India, its extractive separation and antioxidant activity

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ABSTRACT

Now a day, the demand of naturally occurring biomolecules in the present society and global market is well known. *Artocarpus lakoocha Roxb*. is an untapped bioresource of resveratrol, a phenolic compound that is used for its anti-aging, cardioprotective and anticancer activities among many others, from North East India. This work evaluates the effect of parameters such as solvent, time, temperature, speed of agitation, solid to solvent ratio and particle size on the extraction yield of resveratrol from this bioresource. Under the optimized conditions of the process parameters stated above, 85,000 mg/kg resveratrol could be extracted from 90,000 mg/kg *Artocarpus lakoocha Roxb*. within 7 h using ethanol as the solvent. Extraction kinetics was estimated using two different kinetic models. The results showed that the extraction process was dependent on diffusional effect inside the sample. Thermodynamic parameters for extraction process were determined and the process was found to be spontaneous. The compound so extracted showed a significant antioxidant activity with IC_{50} value of 53.24 μg/ml. Thus the extracted resveratrol can be used as therapeutic agent.

1. Introduction

Artocarpus lakoocha Roxb. (ALR), a tropical deciduous tree of 30–40 m height, has been used for furniture, timber, feed etc. The plant is under Plantae kingdom and class is Magnoliopsida. Its family is Moraceae, Genus is Artocarpus and species is Artocarpus lakoocha. The plant contains crude protein, fiber and mineral contents (Roate et al., 2011). Aqueous extract of wood of *Artocarpus lakoocha Roxb*. is called 'Puag haad' and is used as an antihelminthic which contains flavonoids and stilbenoids such as resveratrol and oxyresveratrol (Mongolsuk et al., 1957; Suthira et al., 2012).

Artocarpus lakoocha Roxb. is the richest source of resveratrol and found to contain 0.09 g (w/w) as compared with conventional sources such as grapes (1057 μg/100 ml), peanuts (5.1 μg/g), Itadori plants (68 μg/100 ml) (Burn et al., 2002). However this amount may vary in stem bark, leaf and fruits depending on the fertility of the soil. Resveratrol is a phytoalexin and conventionally present in wines (Romero-Peírez et al., 1996), grape juice (Yasui et al., 1997), grapes (Okuda and Yokotsuka, 1996) and grape berry skins (Romero-P et al., 2001). Resveratrol has chemopreventive and antitumor activities (Jang et al., 1997; Surh et al., 1999); it is also known to decrease coronary heart diseases (Hsieh et al., 1999; Pendurthi et al., 1999). Besides these, it acts as an antibacterial, antioxidant, anthelmintic and insecticide. Resveratrol is used as a food and health ingredient (Cho et al., 2006).

Resveratrol can be extracted by various techniques such as solvent extraction and ultrasonication assisted extraction (Cho et al., 2006). Although many solvents can be used for extraction of resveratrol, ethanol is preferred since it is a green solvent. In the extraction process, the effect of process parameters such as solvent type, extraction time, temperature, particle size, solvent to solid ratio need to be optimized. Some of them are studied by other researchers using different sources of resveratrol (Cho et al., 2006; Romero-P et al., 2001).

In an ongoing research programme, we have been studying extraction of resveratrol from a plant species available in unreserved forest of Assam, India. The aim of the work was to establish the kinetics of extraction of resveratrol. We found that the branches of the plant contain approximately 9% resveratrol along with other important compounds such as oxyresveratrol, cellulose and lignin. The present work reports a detailed study on the extraction of resveratrol from branches of *Artocarpus lakoocha Roxb.* The antiox-

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idant property of the extracted compound was also determined in this study.

2. Materials and methods

2.1. Materials

Standard resveratrol (98%), methanol (CH_3OH, 99%), ethanol (C_2H_5OH, 98%), Propanol (C_3H_7OH) and butanol (C_4H_9OH, 99%) were procured from Sigma Aldrich (USA). 1,1-Diphenyl-2-picrylhydrazyl (DPPH, 95% purity) was purchased from Sigma Aldrich. Water used was distilled by Millipore water system throughout the experiments.

2.2. Preparation of sample

Artocarpus lakoocha Roxb. was collected from Jorhat district of Assam, India. The plant was identified by botanist and the specimen was verified in the herbarium. Branches of the plant were cut into very small pieces of ~1 cm^3 prior to and ease the extraction. The pieces were dried in an oven at 50 °C until constant weight and then ground by WILEY MILL. Ground particles were sieved through mesh of standard size to obtain particles of three sizes:150 μm, 355 μm and 500 μm. The moisture content of the samples was 6% and 0.5%, respectively before and after drying.

2.3. Extraction kinetics study

10 g of ground sample was put in a round bottomed three-necked flask (500 ml) containing a predetermined quantity of solvent and connected with a reflux condenser. A thermometer was placed in one side neck, and the other side neck was used for collection of sample during the extraction process. Constant temperature was maintained throughout the experiment using a water bath. To study the effect of temperature on the extraction, three different temperatures (30 °C, 40 °C and 50 °C) were used for total extraction time of 7 h. 5 ml sample was collected every 1 h. The collected samples were filtered through Whattman inorganic Anopore membrane filters and analyzed by ultraviolet-visible (UV–vis) spectroscopy (EVOLUTION 201, Thermo Scientific) method without further processing. The percentage of extraction was determined using calibration curve obtained from standard resveratrol between concentration (x) in the range of 2.41–7.63 mmol/L and absorbance at 306 nm (y) using the regression equation [y = 0.47797x + 0.1677 (R^2 = 0.99)]. The flow diagram of the extraction process is shown in Fig. 1.

2.4. Characterisation of the sample

The extracted resveratrol were characterised by IR, NMR and Mass spectroscopy. IR spectra were recorded on PERKIN Elmer System 2000^{1}H NMR spectra, ^{1}H and ^{13}C NMR spectra were recorded on ADVANCE, DPXBRUKER, 270 MHz NMR spectrometers. Mass spectra were obtained from TRACE GSQ GCMS instrument manufacturing by M/S Thermo Fisher Scientific Austria Ltd. The heartwood of ALR sample before and after extraction were characterised by XRD and SEM. XRD were done using JDX-11P-3A, JEOL, Japan, Morphology of the samples were done by scanning electron microscope (LEO 1427VP, UK) analysis.

2.5. Characterization of the extracted resveratrol and its antioxidant activity

The antioxidant activity of resveratrol was determined after its extraction. The antioxidant activity was determined on the basis of a radical scavenging effect of stable 1,1-diphenyl-2-picrylhydrazyl (DPPH, 95% purity) (Nooman et al., 2008). An ethanolic solution of DPPH (0.2 mmol/L) was prepared in 70% ethanol and kept overnight. Extracted solution of resveratrol was taken in different sample bottles (10 μl, 20 μl, 30 μl, 40 μl, 50 μl, 60 μl, 70 μl, 80 μl, 90 μl and 100 μl and diluted to 1000 μl. In each sample 1 ml of DPPH-ethanol solution was added and kept under dark for 30 min and then the absorbance was measured at 516 nm in UV spectrophotometer using DPPH-ethanol solution as reference. The antioxidant activity of resveratrol was measured in terms of percent inhibition (IC_{50}) calculated by the following equation:

$$\text{Percent(\%)inhibitionofDPPHactivity} = A - B/Ax100$$

Where A = Optical density of the blank
B = Optical density of the sample
All the data were recorded as triplicate and the values were expressed as ±SD.

2.6. Determination of interaction energy

For determination of the interaction energy between solvent and resveratrol, the structures of solvent and resveratrol were optimized using Gaussian 09 software applying DFT considering B3LYP/631G/++/d,p level of theory. The interaction energy between solvents and resveratrol were calculated using the following equation

$(\Delta E_{AB} = E_{AB} - E_A\text{-}E_B)$(Baruahet al., 2015)

3. Theoretical aspects

3.1. Extraction kinetics

The following two models were considered for the extraction of resveratrol.

Model I

According to Hervas et al. (Harvas, 2006) under equilibrium condition, the equation for extraction kinetics is given as follows

$$\frac{dC}{dt} = k(Co - C) \quad (1)$$

On integration Eq. (1) is written as

$$C = Co\left(1 - e^{-kt}\right)$$

$$lnC = \left(1 - \frac{C}{C_O}\right) = -kt \quad (2)$$

In the linearised form Eq. (2) can be written as

$$\ln\left(1 - \frac{C}{C_O}\right) = -kt \quad (3)$$

Model II

The model was proposed by So and Macdonald (So and Macdonald, 1986) considering the extraction process in two steps;

a) Extraction by washing of material at the beginning of extraction.
b) Extraction governed by diffusion process inside the raw material considering two types of diffusion:

Type 1: unhindered diffusion
Type 2: hindered diffusion

Type 1 diffusion occurs in the broken cells of the material, and Type 2 diffusion occurs in the unbroken cells of the material.

Thus, considering the diffusion effect, the concentration (C_t) of extracted compound at time t is given as:

$$C_t = \left(C_c^w\right)\left(1 - e^{-k_w t}\right) + \left(C_c^{dt}\right)\left(1 - e^{-k_{d_1} t}\right) + \left(C_c^{dt}\right)\left(1 - e^{-k_{d_2} t}\right) \quad (4)$$